SPGR Sub project Completion Report

Studies on the Quantitative Trait Loci (QTL) of economic traits in Black Bengal goat

From 1st April, 2010 to 30th June, 2014

Executing Organization

Department of Animal Breeding and Genetics

Bangladesh Agricultural University

Mymensingh-2202

	Submitted to **PIU-BARC, NATP:Phase -1** **BARC complex** **Farmgate, Dhaka-1215**	

June 30, 2014

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Executive Summary

This experiment was carried out in Bangladesh Agricultural University, Mymensingh, Natore and Bandarban Hill district to produce breeding stock of Black Bengal goat and to detect Quantitative Traits Loci (QTL) of economic traits. Two flocks of goats were reared through contact farmers in Natore and Bandarban Hill district respectively. Growth rate, meat yield, litter size and kidding interval were the traits of interest for QTL study.

The foundation stock for production of back cross progeny in Natore consisted of 193 Black Bengal does, 6 Black Bengal bucks and 4 Beetal bucks. The foundation stock for producing breeding stock in Bandarban Hill district consisted of 91 does and 5 bucks of pure Black Bengal goat. 638 pure Black Bengal kids, 304 cross bred kids and 24 back cross kids could be produced in Natore. 268 Black Bengal kids could be produced in Bandarban Hill district. Average growth rate of kids up to weaning was 57.06 $\pm$ 0.89 g/d and 67.38 $\pm$ 1.50 g/d for Black Bengal and crossbred kids respectively in Natore and 92.99 ± 1.53 g/d for Black Bengal kids in Bandarban. Litter size was 2.06 and 1.67 for Black Bengal does in Natore and Bandarban Hill district respectively. Kidding interval was 209 and 226 days for Black Bengal does in Natore and Bandarban Hill district respectively. Dressing percent was 48.18 $\pm$ 0.61%, 51.84 $\pm$ 0.31% and 49.18 $\pm$ 0.51% for yearling Black Bengal bucks in Natore, yearling Black Bengal bucks in Bandarban Hill district and yearling crossbred bucks in Natore respectively. Black Bengal goat in Bandarban Hill district was superior for growth rate and meat yield. Superior Black Bengal bucks have been produced there and were being multiplied for future breeding purpose. Husbandry of goat rearing was also improved resulting in increased number of goat per family.

DNA was extracted from foundation stocks and subsequent generations of goat in Natore. 40 microsatellite markers of variable length was found suitable for the experimental populations and amplified through PCR using the extracted DNA. Sequencing analysis and phenotypic data are in progress towards detection of QTL.

Existing molecular genetics laboratory of the department was modernized through procurements of essential equipment. Number farmers volunteers, 3 MS students and one Ph.D. students have been produced through this project.

SPGR Sub-Project Completion Report (PCR)

1. **Sub-Project title** : Studies on the Quantitative Trait Loci (QTL) of economic traits in Black Bengal goat
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4. **Duration** of the sub-project: From 1st April, 2010 to 30th June,2014
5. **Date of approval** (by the Executive Council/signing of LoA) : April, 2010
6. **Total approved Budget (Taka):** 1,36,23,820.00

Total fund received (Tk): 1,36, 23,820.00

Total fund Spent (Tk): 1,36,23,820.00

Unspent/balance fund (Tk.): None

Reason for the balance: NA

7. **Justification of undertaking the sub-project** :

Bangladesh possesses only one goat breed known as the Black Bengal goat, exotic breeds such as the Sirohi, Beetal and Jamnapari, and crossbred between the Black Bengal goat and exotic goat. Black Bengal goats are found all over the country. They can be classified into 3 sub populations- Bangladesh West (BBW), Bangladesh Central (BBC) and Bangladesh East (BBH) - according to their geographical distribution and genetic structure (Fig. 1.)

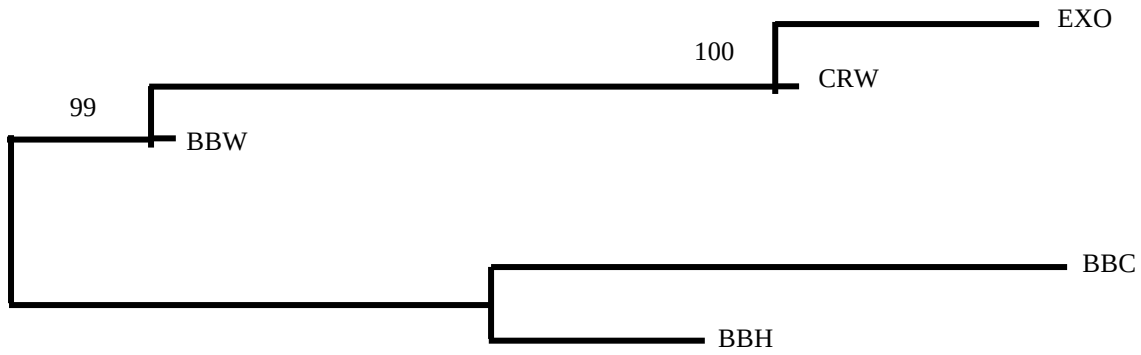

Fig. 1. The NJ topology tree showing the genetic relationships among goat populations of Bangladesh using the Nei (1978) standard genetic distance from 15 microsatellite loci. BBW: Black Bengal goat in the western part; **BBC**: Black Bengal goats in the central part; **BBH**: Black Bengal in the Hilly region including Bandarban, **EXO**: Imported Exotic breeds primarily Sirohi and Beetal; **CRW**: Crossbred goats (Cross between Black Bengal goat and exotic breeds); (Faruque, 2009).

The exotic breeds and crossbred goats are found in the western part of the country and in some specific areas of central and eastern Bangladesh. Sirohi and Beetal bucks are imported by the farmers privately and used mainly for cross breeding purposes. In the absence of any livestock census, it is very difficult to know the exact number of each population or breed. However, Black Bengal goats are clearly predominant (Faruque and Khandoker, 2007). The productivity in terms of growth rate and reproductive efficiency of Black Bengal goat in the Central part of Bangladesh (BBC) has been investigated by a number of scientists. The BBC is a dwarf goat; with an average live weight of approximately 15 kg (at 12 months of age); birth weight ranging from 0.98 to 1.1 kg; maximum growth rate per day is 50 g and dressing percentage IS of 43%. The meat is very delicious and intra muscular fat is low. The does attain puberty at 7 month of age, produce 1st kid at 15 month of age, produce 1.7 litters per kidding. The skin is thin and is suitable for production of fine leather. In fact, Black Bengal goat is famous for it meat and skin quality (Devendra and Owen, 1983; Husain, *at al*. 1998; Jalil, 2003; Faruque and Khandoker, 2007).

Goat was neglected in the past. Government is recently trying to improve this species for increasing food supply as well as income generation. The improvement program is directed towards improvement of husbandry practices as well as genetic improvement. The genetic improvement program should focus on the improvement of economic traits of goat. The traits that can be considered economically important for goat are prolificacy, growth rate, meat quality, skin quality and fitness i.e. disease resistant and survivability.

Traditionally selection of animals for breeding is based on two types of data – pedigrees and phenotypes. Best Linear Unbiased Prediction (BLUP) combines these to generate estimated breeding values (EBVs). This is commonly known as quantitative genetics. In quantitative genetic approach to genetic improvement, the underlying genetic basis of traits has essentially been treated as a 'black box' (Fig. 2a).

Despite this, the substantial rates of genetic improvement that have been achieved is clear evidence of the power of quantitative genetic approaches to selection. This success does, however, not mean that genetic progress could not be enhanced if we could gain insight into the black box of quantitative traits, in particular for traits that are currently difficult to improve. The latter include low heritability traits (litter size, disease resistance) that are difficult to measure (disease resistance), traits that can only be measured on one sex (litter size), traits that are measured late in life (longevity), or traits that require the animal to be slaughtered (meat quality).

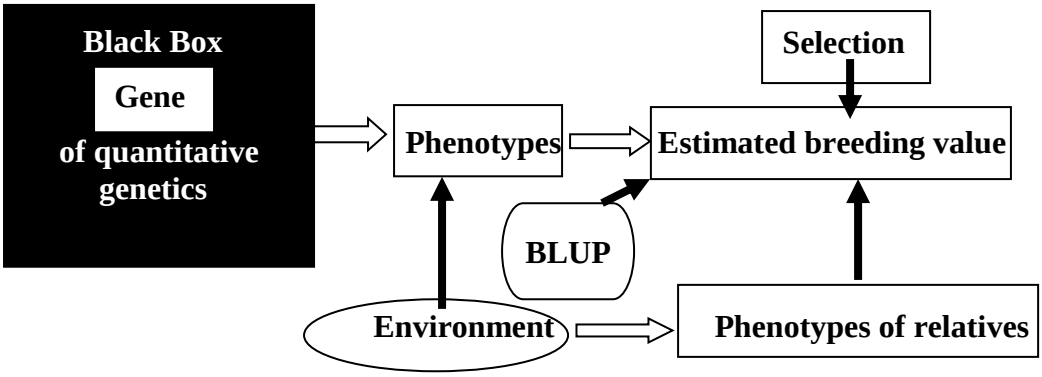

Fig. 2a. Quantitative Genetic selection

A third type of data is based on DNA markers. By being able to study and assess the genetic make-up of individuals at the DNA level through genetic tests, molecular genetics has given us the tools to make those opportunities a reality (Fig. 2b).

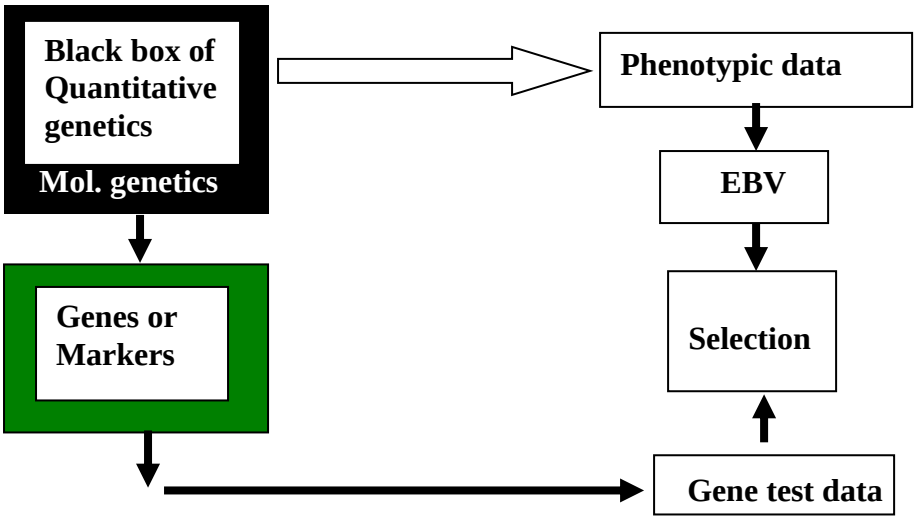

Fig. 2b. Use of molecular data in selection

Molecular data is of interest for use in genetic selection because gene tests have heritability equal to 1 (assuming no genotyping errors), can be done on both sexes and on all animals, can be done early in life, and may require the recording of less phenotypic data (Hayes, 2008; Dekker, 2007). The development of molecular biology techniques and the application of these techniques to farm animals

have progressed rapidly and have opened new vistas for investigators wishing to identify genes that control quantitative traits (Quantitative Trait Loci or QTL).

The aim of QTL analyses is to detect, localize and estimate effects of QTL. The principle of the analyses is to search for non-random associations between phenotypic records and chromosome segments across the genome. Within the segments, the genetic constitution of each animal is deduced from the inheritance of genetic markers. Significant differences in phenotypic expressions between animals with different genetic constitutions indicate the existence of QTL in the studied chromosome segment. In some cases, candidate genes for QTL are known based on information from other populations or other species. If there are known candidate genes, these can be tested directly using polymorphisms within the gene or markers closely linked to the gene. When the aim is to detect unknown QTL, an initial scan of the entire genome has to be performed. The genome scan can show in which chromosome segments QTL are located, but the accuracy of the location is usually low. To increase the precision, and thus improve the possibilities of identifying the QTL, the chromosome segments of interest need to be further studied using other methods, i.e. fine mapping. All phases of QTL mapping involve analyses of quantitative traits that have a complex genetic background and are influenced by environmental factors. Therefore, in addition to the need for genetic marker information, powerful analyses require good phenotypic records from a large number of animals and the use of suitable quantitative statistical methods. Comprehensive genetic linkage maps for the livestock have been developed over the past few years. This has enabled to apply Marker Assisted Selection (MAS) in Livestock. MAS based on QTL are in progress in cattle, pig, sheep and chicken. The genes of interest are *GDF8, CAST, CARNI, and CEBP-α, FecB etc (*Cockett, *et al.* 1994; Abdulkhaliq, *et. al. 2002;* Johnson, et al. *2005; Maddox* and Cockett, *2007).*

Recent investigation on the phenotypes and genotypes of Black Bengal goats in Bangladesh carried out by Faruque (2009) indicates that there is variation in weight and genetic constituents of different populations of Black Bengal goats (Fig. 3 and Fig. 4).

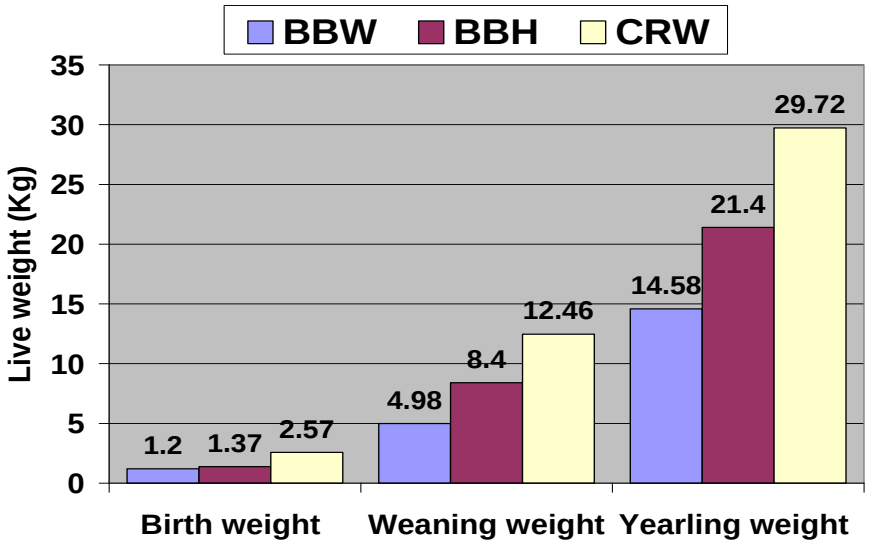

Fig. 3. Growth performances of different genotypes of goat (Legend: BBW- Black Bengal goat in the western part; BBH-Black Bengal goat in the hilly region; CRW-Crossbred between Black Bengal and imported Indian breeds of goat)

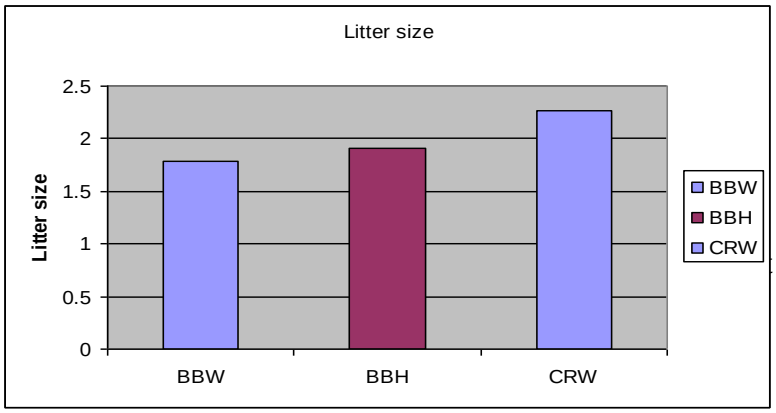

Fig. 4 Litter sizes of different genotypes of goat

This means there is variation within and between populations in Black Bengal goats and there is scope to exploit the genes of economic traits though selection. So, it is justified to identify the genes controlling meat and prolificacy traits of Black Bengal goat for rapid genetic improvement. QTL of goat needs to be identified in order to practice Marker Assisted Selection (MAS) in Black Bengal goat. However these tools, techniques and information are not available for Black Bengal goat in Bangladesh. The present work was, therefore, an attempt to combine conventional breeding with modern biotechnology through study QTL in Black Bengal goat.

8. Sub-project objectives :

i. Production of pure Black Bengal bucks and back cross does
ii. Identifying the Quantitative Traits Loci (QTL) in Black Bengal goat for economic traits
iii. Enhancing the institutional capacity for education and research

9. Methodology followed in conducting research/investigation:

Recruitment of scientific and supporting staffs

The execution and success of any programme depends upon the coordinated work of scientific and supporting staffs. At the beginning of the programme, Scientific Officer, Ph.D. fellow, MS fellow and laboratory technician and supporting staffs i.e. office staffs and field workers were recruited as per Project outline within one month of the project implementation period i.e. within April of 2010.

Selection of experimental sites

Survey was conducted in different Upazila of Pabna, Natore, Rajshahi and Bandarban Hill district for selection of experimental sites. In selecting sites, the following points were considered:

- Availability of desired genotypes of goats in the sites
- Agro-ecological zones and availability of feeds resources
- Communication facilities
- Cooperation of local Livestock Office
- Cooperation of farmers for the proposed program

Finally Gurudaspur and Baraigram Upazila of Natore district and Bandarban sadar Upazila of Bandarban Hill district, that fulfilled the above mentioned criteria, were selected. Nagar Union of Baraigram, Udbaria village of Dharabarisa Union of Gurudaspur Upazila and three villages at Moukhora bazaar at the junction of Baraigram and Gurudaspur Upazila were selected. It may be mentioned that Baraigram is one of the selected Upazila under running NATP programme of Government. The selected sites are shown in Fig. 5 and Fig. 6.

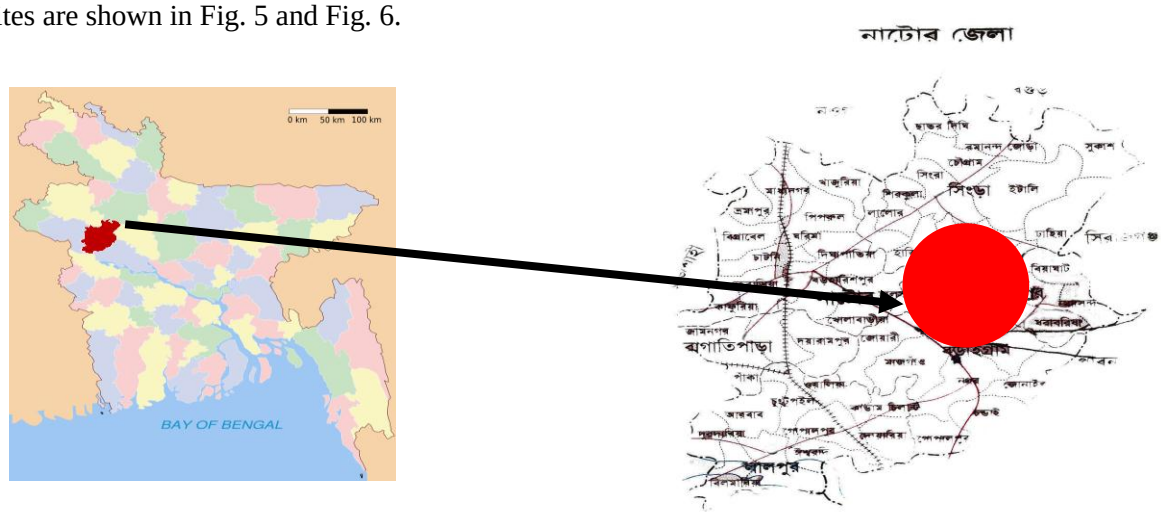

Fig. 5. Selected sites in Natore district indicated by red circle

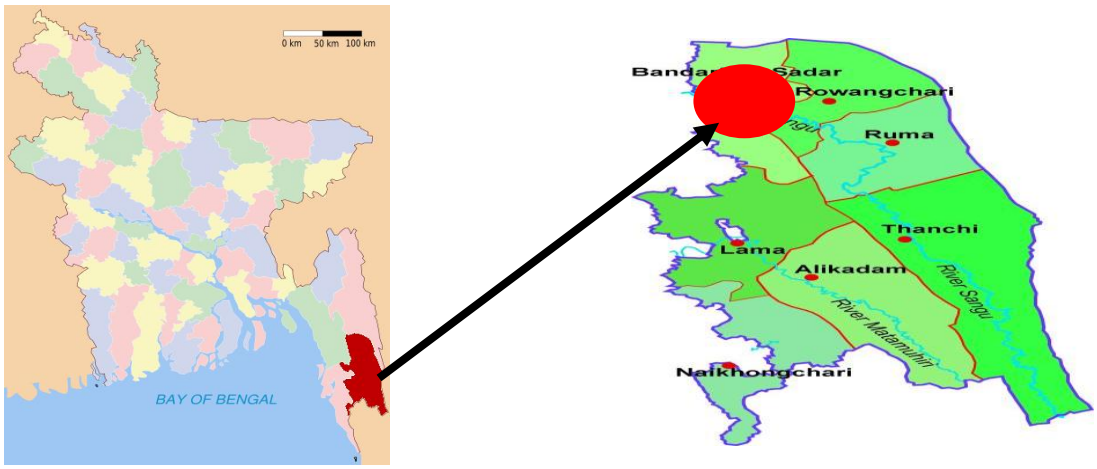

Fig. 6 Selected sites in Bandarban Hill District indicated by red circle

Selection of farmers and experimental goats

An in-depth field survey was conducted in Natore and Bandarban Hill district within the 1st two month of the experimental period (April-May, 2010) in order to get basic information about the socio economic condition of the farmers, livestock demography and husbandry practiced by the goat farmers in the locality. From the result of the survey, targeted farmers and goats (does) were selected. The targeted farmers included in the project agreed to provide their does as per terms and conditions set under this project. A total of 171 farmers with 284 Black Bengal does were selected at the beginning of this study in Nagar village of Baraigram Upazila, Udbaria village, Pam Pathuria village, Khamar Pathuria village and West Naopara village of Gurudaspur Upazila of Natore district; and Krikhonpara, Loxmimohon karabari para, Hansama para of Bandarban Sadar Upazila of Bandarban Hill District as shown in Table 1. These does were the foundation stock for this study and have been termed as G_0 generation.

Table 1. Number of selected farmers and G_0 does

Site	No. of selected farmers	No. of selected BlackBengal does (G_0 goats)
Nagar (Natore)	51	89
Udbaria, (Natore)	39	57
Pam Pathuria Khamar Pathuria West Naopara (Natore)	31	47
Hansamapara Krikhonpara (Bandarban Hill District)	24	38
Loxmimohon karabari para (Bandarban Hill District)	26	53
Total	**171**	**284**

In addition to enlisted does of selected farmers, 6 Black Bengal bucks from of Bandarban Hill District were transferred to Natore units in July 2010 for control mating with G_0 does. The purity and growth of those bucks were ascertained from the pedigree records maintained in Bandarban Hill District since 2007. The Black Bengal goats produced from mating of G_0 does of Natore with Black Bengal buck of Bandarban Hill District were termed as G_1 Black Bengal goats. In July 2011, 4 Beetal bucks were distributed in Natore units for control mating to produce G_2 crossbred goats. All the Beetal bucks were purchased from Rajshahi. The purity and growth of those Beetal bucks were ascertained from the pedigree records maintained in Rajshahi during another study from 2009 to 2010. G_1 Black Bengal does started kidding of G_2 crossbred kids from March 2012 and that continued until March 2013. From January 2013, G_2 crossbred does were mated with pure black Bengal bucks to produce G_3 back cross progenies. The 1st G_3 back cross progeny was produced in September 2013. The foundation stock for production of back cross progeny in Natore, therefore, consisted of 193 selected Black Bengal does, 6 Black Bengal bucks and 4 Beetal bucks. The foundation stock for producing pure seed stock of Black Bengal goat in Bandarban Hill district consisted of selected 91 Black Bengal does and 5 Black Bengal bucks.

Production of pure Black Bengal bucks and does in Bandarban Hill district

The enlisted animals were initially selected from the record of previously run IAEA/BARC project. All the experimental population of goat was pure Black Bengal. The introgression of outside goat weather Black Bengal or exotic breeds was not allowed from the inception of this study and that was maintained until end of the project. At the beginning of the study, the mating system was natural mating system. However, with the progress of the study, superior does and bucks were identified on the basis of growth and prolificacy record. All inferior bucks were castrated. The controlled mating was then implemented among the superior does and bucks. The mating strategy has been presented in Fig 7.

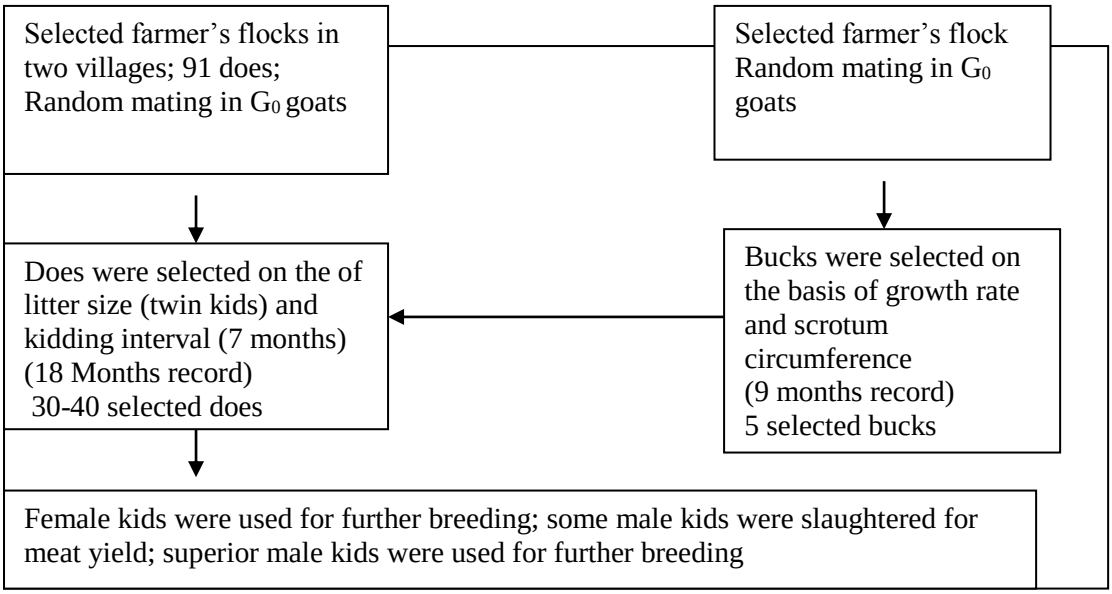

Fig. 7. Mating strategy of Black Bengal goat in Bandarban district

Production of backcross goat in Natore district

The mating strategy for production of back cross goat in Natore has been presented in Fig.8. It has already been mentioned that G_0 Black Bengal does of Natore were mated with Black Bengal bucks of Bandarban Hill District to produced G_1 Black Bengal does (Plate 1a and Plate 1b). 193 G_0 does were mated with 6 Black Bengal buck from the inception of the project to September, 2011. 638 G_1 kids were produced. 4 Beetal Bucks were allowed to mate with the 128 G_1 does from September 1st 2011 to produce G_2 crossbred progenies as per experiment design of this project (Plate 2a and Plate 2b). G_1 does started kidding from March, 2012 and that continued up to the March 2013. 304 G_2 crossbred kids were produced. The G_2 kids when matured were mated with pure Black Bengal bucks to produce G_3 progenies i.e. back cross progenies. Until April 2014, 24 back cross progenies were produced.

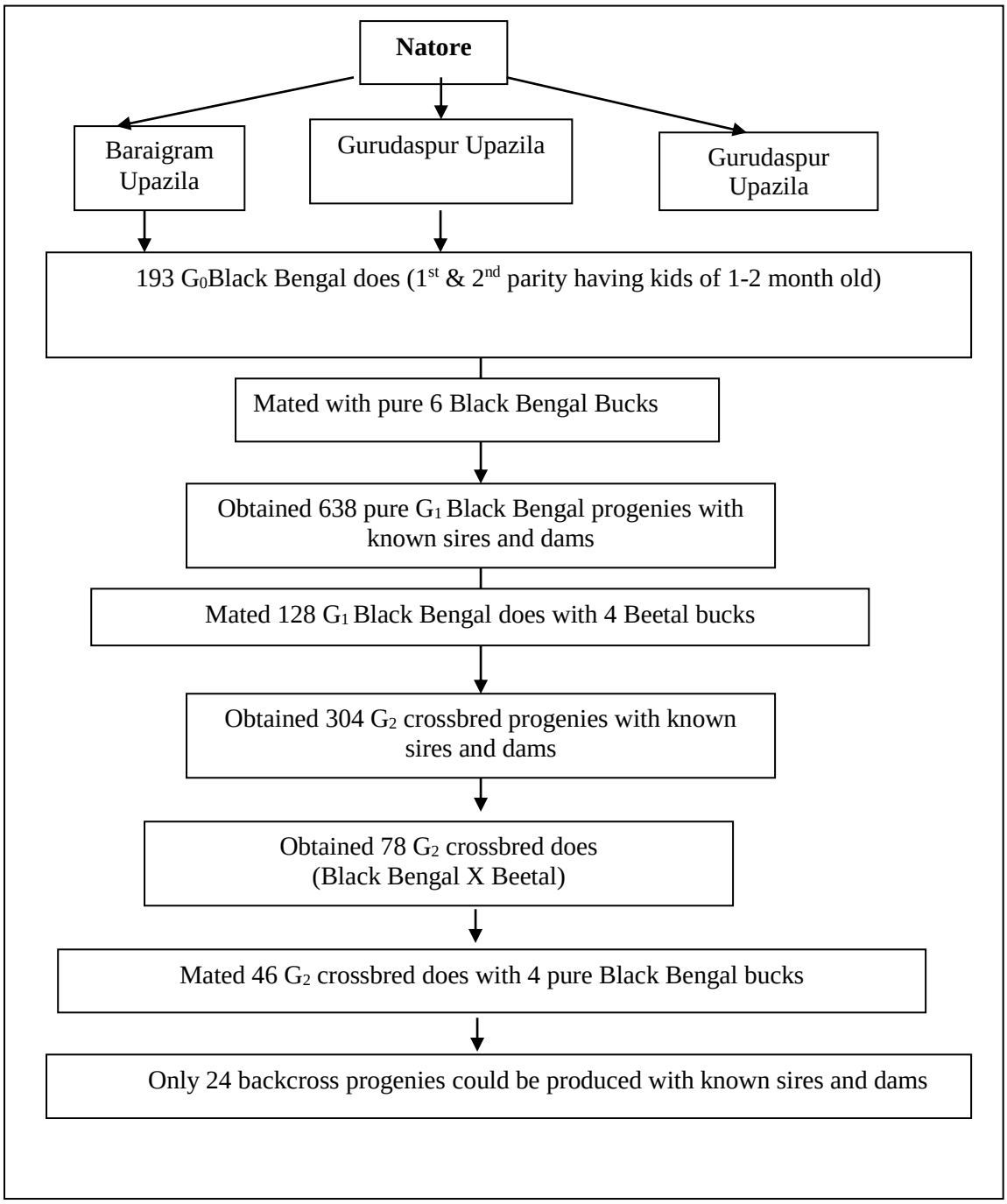

Fig.8. Production of backcross goats in Natore

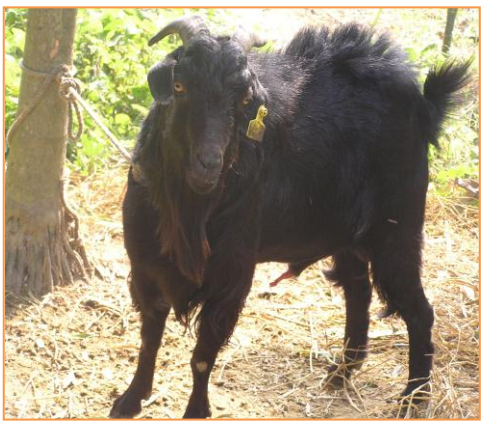

Plate 1a. A G_0 Black Bengal buck

Plate 1b. A G_0 Black Bengal doe

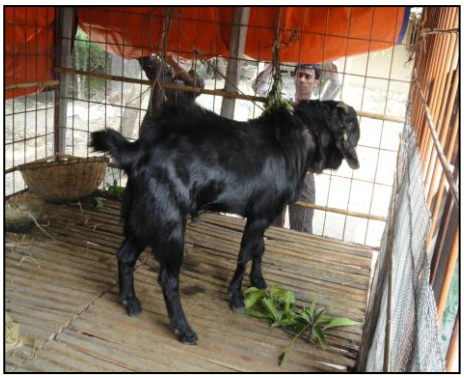

Plate 2a: A Beetal buck

Plate 2b: A G_1 Black Bengal doe

Management of experimental goats

In Natore

All the G_0, G_1, and G_2 does were reared by the contact farmers under semi intensive system following traditional management system. The does and kids were allowed to procure their feeds from the naturally available feeds in the day times and kept in confinement in the night time.

The breeding bucks were kept in confinement during the day and night time. In the morning, they were fed wet whole gram @50g/d for Black Bengal bucks and@200g/d for Beetal bucks. During the day time, they were fed grass/Jack fruit leave *at libitum*. The grass were supplied on cut and carry basis. They were also fed concentrate mixture (3 part crushed wheat/maize + 1 part gram pulse/oil cake) 200 g/d d for Black Bengal bucks and@400g/d for Beetal bucks. Exercise of bucks was done twice a day for half an hour. The bucks were allowed to mate naturally to the enlisted does only once or twice per day depending upon the situation.

Routine vaccination against PPR and Anthrax and routine deworming against internal and external parasites were carried out throughout the experimental period. Treatment was done by Registrar Veterinary doctors in case of outbreak of disease. The record for vaccination, deworming and treatment against incidental diseases were enlisted in the record sheet

In Bandarban Hill district

The management practiced for the experimental flock in Bandarban Hill district was semi-intensive and traditional. The goats browsed in the forest in the day time in most cases (Plate 3). In some areas where forest was thin and scanty, goats grazed in the field. Tethering of goat was uncommon in Bandarban Hill district. Animals were kept confined in bamboo made goat houses in the night time (Plate 4).

Plate 3. A flock of goat browsing in the forest Bandarban Hill district

Plate 4. Common goat house in Bandarban Hill district

Routine vaccination against PPR and routine deworming against internal and external parasites were carried out from the inception of the study. Treatment was done by Registrar Veterinary doctors in case of outbreak of disease. The record for vaccination, deworming and treatment against incidental diseases were enlisted in the record sheet.

Record keeping for production traits (Growth and prolificacy)

In order to study the growth and prolificacy of production traits, record keeping system were introduced from the inception of this study. To ensure accurate record keeping, both farmers and farmer's volunteers were trained. Scientific Officer and Research Fellows supervised the field units to check the records and to make necessary correction. Permanent ear tags with an identification number were set to each does and each buck for proper identification and accurate record keeping of the animals. The following traits were studied in each generation to meet the project objectives:

i. Birth weight of kids
ii. Weight of kids at three months representing weaning weight
iii. Litter size
iv. Kidding interval of does
v. Meat yield

To obtain those parameters mentioned above, field records and laboratory analysis were combined. Birth weight for all kids (including male and female) were taken directly using a digital balance. Three month weight of kids was taken using bath room balance (Plate 5). The weight of record keeper was taken first using bath room balance. Then the weight of record keeper and kids were taken together using the same bath room balance. The weight of kids (in kg) was calculated as difference between Weight of record keeper and kids taken together and weight of record keeper.

Plate 5. Use of bath room balance for weighing kid/goat

Litter size of the does was found out from the record of parturition of does directly. Kidding interval was calculated in days as the differences between two subsequent kidding of doe. To obtain those parameters accurately, the following records were maintained from the inception of this study:

i. Goat Owner, Ear tagging of goat, Identification number of goat
ii. Date of birth, sex, birth weight and three month weight of each kid
iii. Date of heat, date of service, buck number and breed of buck used for servicing, date of kidding, and litter size of each doe.

Analysis of meat

Carcass yield and meat quality were studied for analysis of meat. The laboratory in the department and field was renovated with the necessary equipment like freezer, refrigerator, ice box, water bath, grinder etc. taking financial support from IAEA funded project. A number of goats from Natore and Bandarban Hill district of age between 9-11 months covering does, castrated males and bucks were slaughtered to determine the meat yield of goat. Samples of meat from representative animals were collected to study the meat quality**.**

In absence of any grading system for carcass yield and meat quality of goat in Bangladesh, attempt was made to grade carcass yield and meat quality of experimental goat of this project as shown in Plate 6, Plate 7 and Plate 8.

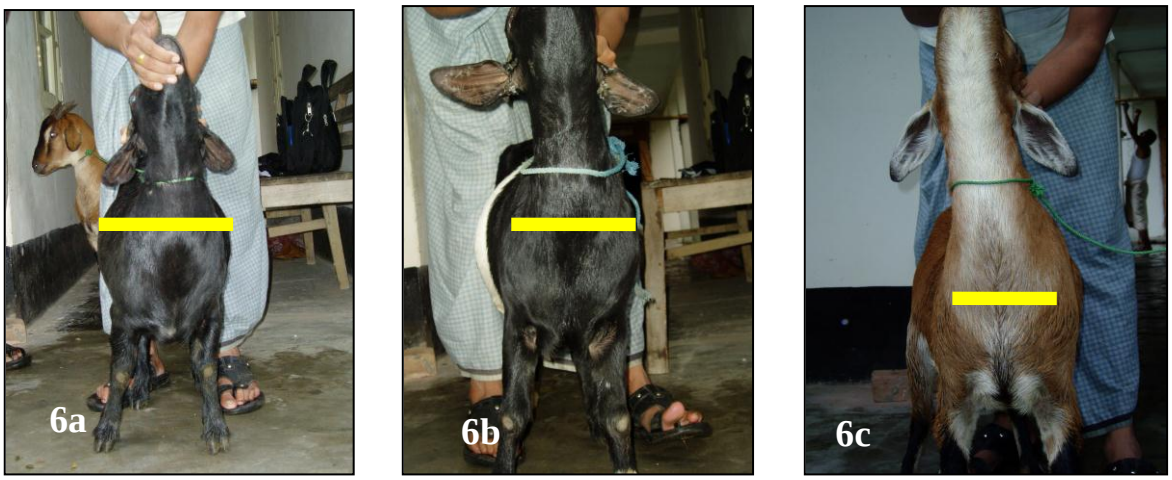

Plate 6: Front view of goat used as meat animal. 6a- Best (Grade A), 6b - Better (Grade B), 6c - Good (Grade C)

Plate 7: Rear view of goat used as meat animal. 7a- Best (Grade A), 7b - Better (Grade B), 7c - Good (Grade C)

Plate 8a: Carcass of A grade goat

Plate 8b: Carcass of C grade goat

The following slaughtering technique was used:

- Goats were weighed and then fasted for 6 hours with free access to water and weighed again immediately prior to slaughter
- Goats were slaughtered after halal method
- After complete bleeding, the slaughtered animals were skinned.
- The head was detached at the atlanto-occipital joint, and the fore and hind cannons were removed at the knee and hock joint respectively.
- All abdominal and thoracic organs were removed and weighed.
- Internal fat deposited on the top of the kidneys (perinephric fat) and around gastro-intestinal tract (gut fat) were separated and weighed.
- The tail was cut off at its articulation.
- Tail, genitalia and cannons were excluded.

The hot carcass yield (HCY) and the alimentary tract (gut) without its content were weighed. The empty live weight (ELW) was computed as the difference between slaughter weight and weight of digesta content. Carcass and non carcass components were weighed immediately after slaughter. Lungs, trachea and heart were weighed as one piece and designated as pluck. Non-carcass components included head, skin, feet, digestive tract, liver, spleen, pancreas and pluck. Weight of digestive contents was computed as the difference between full and empty digestive tract.

Dressed carcasses were weighed within 1 h (hot carcass weight). Dressed carcasses chilled for 24 h at 4^0 C and weighed again (cold carcass weight)The dressed carcass was split into fore and hind quarters and loin eye area (cm^2) was recorded on the cut surface of M. longissimus dorsi at the interface of 12th and 13th rib, on both sides of the carcass. M. longissimus dorsi was collected within 20 min of slaughter, trimmed of fat and chilled at 4^0C and analyzed for meat quality traits. Carcass yield was determined as dressing percent on hot carcass basis, chilled carcass basis and empty stomach basis**.** Proportion edible and non edible parts of animals as well as proportion of different portion of carcass were also determined. Some of those activities have been shown in Plate 9.

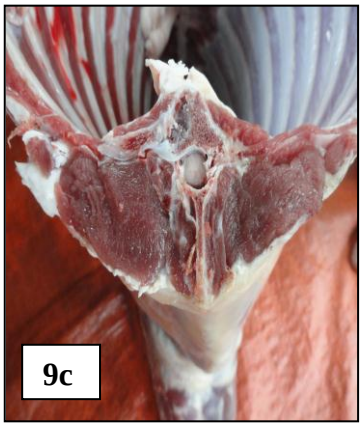

Plate 9a: Flaying of skin; Plate 9b: Carcass measurement; Plate 9c: Rib eye area of carcass

Meat quality was judged by proximate component analysis, Ph of meat, water holding capacity, sensory test of cooked meat.

Identifying the Quantitative traits loci

A full genome scan for QTL includes five steps. These are (i) Choice of a mapping population, (ii) Collection of phenotype data, (iii) Genotyping: In livestock, short tandem repeats or microsatellites are currently the markers of choice as they are highly polymorphic and more than a thousand of them are available in most species, (iv)) Setting up a genetic model for QTL, and (v) Drawing statistical inference from data.

The experiment is being done on the basis of back crossing method as shown in Figure 6. The experiment will be completed through 4 steps:

i. ***Production of experimental populations***: The 193 Black Bengal does, 6 Black Bengal bucks and 4 Beetal bucks and 11 Black Bengal bucks Natore was the foundation stock and their subsequent generations in G_1, G_2 and G_3 were the experiment populations for this study. 638 G_1 (Pure Black Bengal), 304 G_2 (cross bred) and 20 G_3 (back cross) progenies could be produced so far.

ii. ***Collection of phenotype data*:** Data on the phenotypes viz., growth rate, litter size and kidding interval, carcass quantity were collected from the above mentioned populations. The following phenotypic data were, therefore, recorded:

Growth and meat traits
Birth weight, weaning weight, meat yield

Prolificacy traits
Litter size, kidding interval

iii. ***Genotyping and QTL detection*****:** Genotyping was based microsatellites markers. The procedures for genotyping involved blood collection, DNA isolation and quantification, primers selection and PCR amplification, sequencing, alleles determination and genome scanning.

Blood collection

Blood samples of all bucks (6 Black Bengal and 4 Beetal), 160G_0 does, 128G_1 kids, 78G_2 kids, and 20 G_3 kids was collected. Blood was collected from jugular vein using venoject tubes coated with EDTA. 10 ml of blood was collected in each venoject tube. The collected blood was kept in cooling box and was carried to Animal Genetic laboratory, Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh and transfer to refrigerator in which blood samples were preserved at 4°C to 8°C. DNA was extracted immediately.

DNA extraction

DNA was extracted using DNA extraction kit of Qiagen viz., QIAamp® DNA mini kit and DNA extraction kit of Promega viz., Genomic DNA purification kit. For both kits, whole blood was used for DNA isolation.. The protocol for Qiagen kit has been described below:

- 20µl QIAGEN proteose was added in to the bottom of a 1.5 ml microcentrifuge tube.
- 200µl whole blood was added to that microcentrifuge tube.
- 200µl of buffer AL (supplied in the kit) and mixed by vortex for 15 second.
- The mixture was incubated at 56^0C for 10 minutes.
- After incubation, the mixture was centrifuged briefly in same microcentrifuge tube to remove drops from the inside of the lid.
- 200µl of ethanol (96%) was added to the sample and mixed by vortex for 15 second.
- The mixture was then transferred to QIAamp mini span column in a without wetting the rim. The cap of the tube was closed and centrifuged at 6000xg (8000rmp) for 1 minute. QIAamp mini span column was placed in a clean 2 ml collection tube (provided) and the filtrate was discarded.
- The QIAamp mini span column was carefully opened and 500µl of buffer AW1 (provided) without wetting the rim. The cap of the column was closed and centrifuged at 6000xg (8000rmp) for 1 minute. QIAamp mini span column was placed in a clean 2 ml collection tube (provided) and the filtrate was discarded.
- The QIAamp mini span column was carefully opened and 500µl of buffer AW1 (provided) without wetting the rim. The cap of the column was closed and centrifuged at 20000xg (14000rmp) for 3 minute.
- The QIAamp mini span column was placed in new 2 ml collection tube and the old collection tube contain filtrate was discard. Centrifuged at full speed for 1 min. The QIAamp mini span column was opened and 200 µl buffer of AE (provided) was added.
- The substrate (containing DNA) was incubated at room temperature (25^0C) for 1 minute and then centrifuged at 6000 x g for 1 minute.

- The substrate (containing DNA) was preserved at -20^{0}C for quantification and further processing.

Similarly, DNA was also extracted using DNA extraction protocol of Promega, USA kit ReliaPrepTM Blood gDNA Mini System (250 preparations) vide Catalog N0. A1125.

DNA Quantification

The extracted DNA was quantified using Nano Drop spectrophotometer. Spectrophotometer was calibrated for DNA/RNA analysis. One µl of DNA sample was added to the cuvette of the machine and DNA was quantified automatically. DNA quantity was measured in µg/µl and quality was measured as the ration of 260/280. Those DNA was accepted for PCR amplification that had a concentration above 40 µg/µl and a ratio between 1.7 to 1.9.

PCR AMPLIFICATION

78 of label and unlabeled primers microsatellites marker were procured. 40 markers were proved suitable for this experimental population. The sequence and annealing conditions have been presented in Table 2. DNA, extracted from all the goats, was amplified using the 40 microsatellite markers. 10 µl PCR mixture was made for each marker. The annealing temperature for each marker has been presented in

Table 2. List of some markers that were found positive in PCR across the populations

Name of Markers	Sequence of primers	Primer length	Annealing temp.
ILSTS - 005	GGAAGCAATTGAAATCTATAGCC –F TGTTCTGTGAGTTTGTAAGC -R	23 20	55^{0}C
ILSTS - 011	GCTTGCTACATGGAAAGTGC –F CTAAAATGCAGAGCCCTACC -R	20 20	58^{0}C
ILSTS - 029	TGTTTTGATGGAACACAG –F TGGATTTAGACCAGGGTTGG -R	18 20	55^{0}C
CSRD 247	GGACTTGCCAGAACTCTGCAAT –F CACTGTGGTTTGTATTAGTCAGG -R	22 23	58^{0}C
ETH 10	GTTCAGGACTGGCCCTGCTAACA –F CCTCCAGCCCACTTTCTCTTCTC - R	23 23	55^{0}C
IRNA 063	GACCACAAAGGGATTTGCACAAGC -F AAACCACAGAAATGCTTGGAAG -R	24 22	58^{0}C
MAF 65	AAAGGCCAGAGTATGCAATTAGGAG–F CCACTCCTCCTGAGAATATAACATG- R	25 25	58^{0}C
MAF 70	CACGGAGTCACAAAGAGTCAGACC –F GCAGGACTCTACGGGGCCTTTGC - R	24 23	65^{0}C
MAF 209	GATCACAAAAAGTTGGATACAACCGTG –F TCATGCACTTAAGTATGTAGGATGCTG -R	27 27	55^{0}C

SRCRSP 23	TGAACGGGTAAAGATGTG – F TGTTTTTAATGGCTGAGTAG -R	18 20	55^0C
SRCRSP 5	GGACTCTACCAACTGAGCTACAAG –F TGAAATGAAGCTAAAGCAATGC - R	24 22	55^0C
SRCRSP 7	TCTCAGCACCTTAATTGCTCT – F GGTCAACACTCCAATGGTGAG - R	21 21	55^0C
SRCRSP 15	CTTTACTTCTGACATGGTATTTCC –F TGCCACTCAATTTAGCAAGC - R	24 20	55^0C
OarAE 54	TACTAAAGAAACATGAAGCTCCCA–F GGAAACATTTATTCTTATTCCTCAGTG- R	24	58^0C
P19 (DYA)	AACACCATCAAACAGTAAGAG – F CATAGTAACAGATCTTCCTACA - R	21 22	55^0C
TGLA53	GCTTTCAGAAATAGTTTGCATTCA –F ATCTTCACATGATATTACAGCAGA -R	24 24	55^0C

Collection of blood samples and DNA isolation from all possible and available individuals of desirable populations has been completed by the end of April. Markers were tested in the mean time. Final PCR amplification will be finished by 31st May, 2014. It is expected the sequencing and allele detection will be done in June, 2014. The data will be put into the software to preferably Gene mapper, Join map version ®4.1 or MapQTL ®6.0 to detect the length of genome, interval mapping and QTL.

iv. ***Statistical inference***:

The statistical testing for QTL will be performed at marker loci (single marker analysis) as well as in intervals between markers (interval mapping). Multiple testing across the genome will be considered when setting significance thresholds. Parameters will be estimated in the most likely positions for QTL by regression, ML, BLUP-based methods.

Modernizing of existing molecular genetics laboratory of the department

The existing molecular biology laboratory of the department was renovated by procuring some valuable equipment from the project fund. The equipment was Nano drop spectrophotometer, Gel Documentation machine, Rotor centrifuge machine and Microtome machine**.** Nano drop spectrophotometer is used for quantification of DNA, RNA and Protein, Gel documentation machine is used for visualization of DNA in Genomic and PCR products, Microtome machine is used in cutting fine section during slide preparation for histological study, Swing bucket rotor centrifuge machine etc (Plate 10).

Plate 10a. Microtome machine

Plate 10b. Nano drop Spectrophotometer

Plate 10c. Gel Documentation machine

Plate 10d. Rotor centrifuge machine

Workshop, training and group meeting of farmers

Group discussion and farmer training were organized in each village to make this program effective and fruitful (Plate 11 and 12).

Plate 11: Group discussion with farmers in Natore

Plate 12: Group discussion with farmers in Bandarban Hill district

In addition to group discussion, a manual on record keeping and preventive measure of goat was printed. This was distributed to the farmer volunteers and enlisted farmers of each village (Plate 13).

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Plate 13. Distribution of manual on profitable goat rearing to a farmer in Pam Pathuria village

A daylong national work "Studies on the QTL of economic traits in Black Bengal goat" was organized on December 9, 2012 in the auditorium, Bangladesh Agricultural Research Council, Dhaka

The daylong workshop was divided into 4 sessions. These were Opening session, Technical session –I, Technical session –II and Discussion & recommendation session**.** A total of 80 livestock scientists and experts from different universities including Bangladesh Agricultural University, Department of Livestock Services, Bangladesh Livestock Research Institute, Bangladesh Agricultural Research Council and NGO (BRAC) participated into the workshop. Electronic media personnel also participated into the workshop.

The Opening session was chaired by Prof. Md. Mohsin Ali**,** Director, BAURES, Bangladesh Agricultural University, Mymensingh. **Md. Abdul Latif Biswas**, Honourable Minister, Ministry of Fisheries and Livestock, Government of the People's Republic of Bangladesh and Mr**.** Md. Asraf Ali, Director General, Department of Livestock Services, Bangladesh were the chief guest and special guest of workshop respectively. Prof. Dr. Md. Omar Faruque**,** Principal Investigator of the project gave the Welcome address while Prof. Dr. Md. Ruhul Amin, Co-Principal Investigator of the project gave the Vote of Thanks (Plate 14). The Opening session started at 10.00 am and closed at 11.00 am.

Technical session –I was chaired by Mr. Md. Asraf Ali, Director General, Department of Livestock Services, Dhaka**.** In this session, Professor Dr. Md. Omar Faruque, Principal Investigator of the project presented the key note paper and addressed on **"**Application of Biotechnology in goat breeding: Experience of the experiments in Bangladesh". He highlighted the goal and aims of this project, discussed the methodology of the project and described the results and outcomes of the project especially the benefits of contact farmers of this project by goat rearing. This session started at 11.00 am and closed at 1.00 pm.

Technical session –II was chaired by Dr. Md. Kalekuzzaman Akond, Member Director (Livestock), Bangladesh Agricultural Research Council, Dhaka. In this session**,** Prof. Dr. Md. Ruhul Amin, Co-Principal Investigator of the project addressed on phenotypic characteristics of experimental goat populations in Natore and Bandarban district. He described the phenotypic characteristics of pure Black Bengal goats and crossbred goats so far produced in this project. Then three contact farmers from Bandarban and Natore district described their experience on goat rearing especially the benefit of goat rearing following training and instruction given to them from this project. Technical session –II started at 2.00 pm and ended at 4.00 pm.

Discussion and recommendation session started at 4.00 pm under the chairmanship of Prof. Md. Mohsin Ali**,** Director, BAURES, Bangladesh Agricultural University, Mymensingh. A number of participants took part in the discussion.

Plate 14a. Opening session of day long workshop

Plate 14b. Participants of day long workshop

10. Results and discussion

Collection of base line information

The farmer's demography, cultivated land possessed by the framers, literacy status, number and types of livestock in the selected sited as estimated from survey have been presented in Table 3 and Table 4.

Table 3 Demography, cultivated land and literacy status of farmers in the selected sites

Site	Family member (No.)	Cultivated land Acre	Literacy (No.)
Nagar, Baraigram (N= 73)	4.30 ±. 0.22	3.30 ±. 0.67	1.30 ±. 0.18
Udbaria, Gurudaspur (N= 103)	4.71 ±. 0.18	3.19 ±. 0.54	0.64 ±. 0.08
Panpathuria, Moukhora (N=50)	4.20 ±. 0.18	2.06 ±. 0.50	0.28 ±. 0.08
Hansamapara, Bandarban sadar (N = 20)	4.05 ±. 0.10	0.38 ±. 0.06	1.01 ±. 0.22

Table 4 The number and type of livestock reared in the selected sites*

Site	Cattle	Goat	Chicken	Duck
Nagar, Baraigram (N= 245)	0.96 ± 0.08	3.03 ± 0.12	4.33 ± 0.49	1.65 ± 0.25
Udbaria, Gurudaspur (N= 103)	0.85 ± 0.14	4.12 ± 0.19	5.49 ± 0.45	1.35 ± 0.17
Panpathuria, Moukhora (N=50)	1.12 ± 0.08	2.71 ± 0.11	5.39 ± 0.35	1.25 ± 0.15
Hansamapara,** Bandarban sadar (N=20)	2.40 ± 0.29	4.70 ± 0.38	3.90 ± 0.58	-

*Horse and pig were absent in the selected sites of Natore district; sheep and buffaloes were scare
** Sheep, buffalo and horse were absent in the selected sites of Bandarban Hill district. Pigs were present in most of the household in limited number

The study indicated the distribution and utilization in different ecological zones and in different human communities of the country. Natore is flood fed area and most of the people are Muslim. So, sheep and pig were absent there. On the other hand, Bandar Hill district is the hilly region of the country and most of the people are Buddhist. So pig was common there in addition to cattle, goat and chicken. However, the lack of horse is an exception as this species is very common in the hilly area of the world.

a. Production of pure Black Bengal goat in Bandarban

The production of breeding stock in Bandarban was actually initiated in 2007. After making several attempts in different villages, the initiative was finally concentrated in Hansamapara and Krikhonpara villages in Bandarban Sadar Upazilla under BARC funded previous project in 2007 and present SPGR funded project. After making several generations of random mating and selection for birth weight and weaning weight, the performances of present Black Bengal goat population in those two villages has improved (Plate 15).

Plate 15. A flock of seed stock of Black Bengal

The performance of this population in Bandarban Hill district has been described below:

- The birth weights of kids ranged from 0.8 to 2 kg with a mean of 1.06 ± 0.01 kg (n = 268).
- Weights of kids at 3 months ranged from 5 to 12 kg with a mean of 9.38 ± 0.14 kg (n = 150).
- The average daily gain ranged from 44.44 to 143.33 kg with a mean of 92.99 ± 1.53 g/d.
- The litter size of does ranged from 1 to 4 with a mean of 1.67 ± 0.05 (n = 103 does with 200 kidding).
- The kidding interval of does ranged from 125 to 419 days with a mean of 226.35 ± 10.01 days (n = 57).

Initially the performance of BBH in 2007 was lower than the present stock. The performance of BBH in 2007 has been given below:

- The birth weights of kids ranged from 0.8 to 1.5 kg with a mean of 1.01 ± 0.02 kg (n = 155).
- Weights of kids at 3 months ranged from 5 to 9 kg with a mean of 7.25 ± 0.16 kg (n = 125).
- The litter size of does ranged from 1 to 3 with a mean of 1.70 ± 0.06 (n = 80 does with 170 kidding).
- The kidding interval of does ranged from 150 to 410 days with a mean of 230 ± 12.02 days (n = 55).

From the above results, it can be concluded that the present stock of Bandarban Hill district is superior in performances.

Main characteristics of this population are:

- This population is genetically pure Black Bengal goat.
- The kids at 3 months of age of this population are the heaviest kids within Black Bengal goat in Bangladesh (Table 5). The selected bucks have yearling body weight of 30 kg.
- There was no outbreak of infectious disease like PPR, Anthrax and FMD in this population in the last five years.

The comparative phenotypic performances of this population and other populations of Black Bengal goats in Bangladesh have been presented in Table 5.

Table 5. Phenotypic performances of different populations of Black Bengal goats

Population	Birth weight (kg)	Weaning weight (kg)	Litter size (no.)	Kidding interval (days)	Reference
BBH	1.06	9.38	1.67	226	Present study
BBC	0.98	4.86	1.93	220	Husain *el al.* 1996
BBC	1.08	5.22	1.52	-	Haque *et al.* 2011
BBC	1.23	4.81	1.35	-	Hasanat *et al.* 2003
BBC	-	-	-	223	Ali *et al.* 1973
BBW	1.24	6.36	2.06	209	Present study

Legend - BBH: Black Bengal goat of this experiment in Bandarban, BBW: Black Bengal goat of this experiment in Natore, BBC: Black Bengal goat of central part of Bangladesh mainly of Mymensingh district.

From Table 5, it appears that birth weight of BBH population is1.06 kg, weaning weight is almost double than the Black Bengal goat of central part of Bangladesh (4.81). It may be mentioned here that weaning weight represents the maximum genetic effect of an animal. After weaning, the genetic effect is minimized and environmental effect is maximized. The weight of bucks was also high. The breeding bucks weigh about 30 kg at one year of age. Litter size was also at the upper limit as compared to BBC population. However, kidding interval was little higher, but within the desired limit i.e. about 7 months. So this satisfies the basic two requirements for rearing Black Bengal goat for meat purpose i.e. growth and prolificacy. Nezowa, *et al.* (1984) and (Faruque, 2009) mentioned that BBH goats are the largest in size among the three population of goats in Bangladesh. The higher birth weight and weaning weight

might be due this factor. However, how much is the genetic factor and how much is the environmental factor for such different needs to be invested further.

b. **Performance of Black Bengal goat, crossbred goats and back cross goats in Natore as part of production of back gross goat**

The Black Bengal goats of Natore consist of G_0 and G_1 populations. The reproductive and growth performances of these two generations have been presented in Table 5, Table 6 and Table 7.

Table 6. Gestation period, litter size and kidding interval of G_0 goat in Natore

	N	Minimum	Maximum	Mean	Sd. Error
Gestation period (Day)	368	112	165	146.84	0.38
Litter size (No.)	Doe- 180 Kidding- 303	1	4	2.05	0.46
kidding interval (Day)	146	156	299	209.14	2.791

Table 7. Age at puberty and Gestation period of G_1 goat in Natore

	N	Minimum	Maximum	Mean	Sd. Error
Age at puberty (Day)	85	105	398	269.84	11.91
Gestation period (Day)	73	129	162	145.24	0.79

Table 8. Birth weight, weaning weight and daily growth rate of Black Bengal goat (G_0 and G_1 combined) kids in Natore

	N	Minimum	Maximum	Mean	Sd. Error
Birth weight (kg)	622	0.5	2.55	1.24	0.01
Weaning weight (kg)	435	3.0	13.0	6.36	0.01
Daily growth rate (g/d)	435	11.0	193.33	57.06	0.89

6 bucks were used in producing G_1 kids in Natore. There was variation in birth weight of kids within and between groups of bucks as evidences in and Table 8 and Fig.9. The very low birth weight of kids in buck- 4 may be due to very low number of kids as compared to others bucks.

Table 9. Birth weight of G_1 kids of different Black Bengal buck groups in Natore

	N	Minimum	Maximum	Mean	Sd. Error
Kid Birth wt., total kids(Kg)	97	0.5	1.9	1.274	0.2796
Male kids(Kg)	50	0.8	1.9	1.354	0.2597
Female kids(Kg)	47	0.5	1.9	1.189	0.2776

Kids of Buck 1 (Kg)	16	1.0	1.6	1.306	0.1526
Kids of Buck 2 (Kg)	32	0.7	1.7	1.325	0.2185
Kids of Buck 3 (Kg)	33	0.5	1.9	1.294	0.3427
Kids of Buck 4 (Kg)	2	0.7	0.9	0.800	0.1414
Kids of Buck 5 (Kg)	14	0.8	1.8	1.143	0.2901

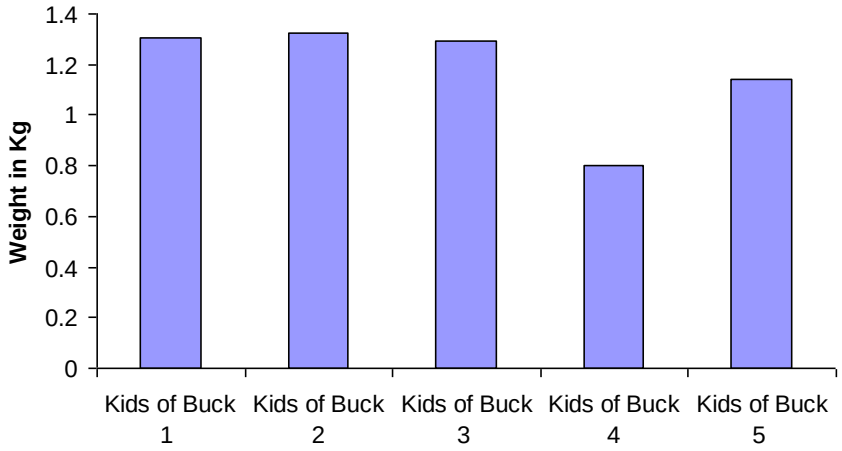

Fig. 9. Birth weight of kids of different sire groups in Natore

Crossing breeding of Black Bengal does with Beetal buck resulted in production of a number of crossbred goats. 304 crossbred G_2 progenies could be produced. The crossbred goats attained puberty at 299.06 days on an average with a range from 169 days to 405 days. This is slightly higher than that of pure Black Bengal goats. Pure Black Bengal goats attained puberty at 269.84 days on an average with a range from 105 days to 398 days. The weight and growth rate of crossbred kids has been presented in Table 9.

Table 10. Birth weight, weaning weight and daily growth rate of G_2 (crossbred) kids in Natore

	N	Minimum	Maximum	Mean	Sd. Error
Birth weight (kg)	304	0.6	4.03	1.46	0.03
Weaning weight (kg)	202	3.8	15.0	7.58	0.15
Daily growth rate (g/d)	202	32.22	142.02	67.38	1.50

The mating of G2 Crossbred does with pure Black Bengal buck resulted in production of back cross progenies. Only 24 back cross progenies could be produced until March 2014. Only body weight of these

kids could be recorded and analyzed. Table 10 shows the comparative birth weight and growth rate of pure Black Bengal, cross bred and back cross goats in Natore.

Table 11. Weight and growth rate of Black Bengal, crossbred and back cross kids in Natore

Parameter	*Black Bengal goat*	*Croos bred goat*	*Backcross goat*
Birth weight (kg)	1.24± 0.01	1.46 ± 0.03	1.44 ± 0.08
3 month weight (kg)	6.36 ± 0.01	7.58 ± 0.15	-
Growth rate (gm/day)	57.06 ± 0.89	67.38 ± 1.50	-

The birth weight of kids in the BBW population in Natore was the highest as compared to other two populations Viz., BBC and BBH. The highest birth weight of kids in Natore might be for highest body size as mentioned earlier. However, the highest weaning weight in BBH goat as compare to BBC and BBW populations might be for maternity effect as well as environmental effect. Most of the studies on growth rate of Black Bengal goat have been done on the BBC population as mentioned by Husain, *et. al.* (1998), Haque, *et al. (*2011), Mohon, *et al.* (2013) and others. They have mentioned lowest growth rate of Black Bengal goat in BBC population. This might be for environmental effect. However, to make the final conclusion, studies should be done taking all the genotypes in same environment. Singh (1989) , Singh and Sengar (1990) reported the same birth weight of kids in Black Bengal goat of West Bengal ranging from 1.0 to 1.23 kg. They report lower weaning weight of kids (4.23 kg) than the present study (6.36 to 9.38 kg). There was variation in birth weight of kids in different sire groups. That was due to genetic effect. The birth weight and weaning weight of crossbred kids in Natore were higher than the pure Black Bengal goat. That was due to heterosis effect. The reproduction performance of Black Bengal does was quite satisfactory. They attained puberty with 8 months and kidding interval was around 7 months. However, Crossbred does attained at higher age that around 11 months. Some of the does even attained puberty at 15 months and some did not show any estrus

symptom even after treating with hormone. Such performance of beetal crossbred has been reported by Singh. *et al.* (1983). According Acharya (1982) the performance of Beetal in farmers' flocks was: age at first kidding: 559 days: kidding interval: 357 days. Under farm conditions, age at first kidding: 761.87 ± 4.24 days; kidding interval: 368.0 ± 2.44 days; litter size (6, 20): singles: 40.66%; twins: 52.6%; triplets: 6.52%, quadruplets: 0.22% (based on 2 487 kids born). In the present study, the selection of Beetal was on the basis of availability of exotic breed. Onlt Sirohi and Beetal were available in the local market and The Beetal buck procured had some pedigree information. However, due to low reproduction performance of crossbred does in the semi intensive management system, required number of back crossbred progeny could not be produced and that hampered the QTL detectation later on.

Carcass analysis

The hot carcass yield (on full stomach basis) of doe, castrated male and buck of Black Bengal goat of 9 to 11 month of age in Natore and Bandarban has been presented in Table 11 and Table 12. The dressing% ranged from 36.16 to 50.32% for randomly selected population of Black Bengal goat in Natore. The hot carcass yield and dressing% was highest for buck and lowest for the doe. On the other hand, hot carcass yield of the bucks of Bandarban for the same age ranged from 50 to 55%. This variation is certainly statistically significant. The dressing% reduced slightly on chilled carcass basis. However, it increased 10 to 15% on empty stock basis.

Table 12. Carcass yield, dressing% and proximate analysis of different sex group of G_0 goat in Natore

Sex	Live weight (kg)	Hot carcass yield (kg)	Dressing %	Moisture %	Protein %	Ash %
Buck (N= 16)	17.5 ± 1.83	8.42 ± 0.79	48.18 ± 0.61	74.49 ± 0.20	22.06 ± 0.17	1.10 ± 0.07
Castrated male (N = 35)	15.5 ± 0.86	6.75 ± 0.62	43.51 ± 0.61	74.30 ± 0.12	22.00 ± 0.13	1.09 ± 0.12
Doe (N = 29)	14.9 ± 1.03	5.89 ± 0.37	39.85 ± 0.66	74.49 ± 0.20	21.86 ± 0.17	1.22 ± 0.11

Table 13. Carcass yield, dressing% and proximate analysis of Black Bengal goat in Bandarban

Sex	Live weight (kg)	Hot carcass yield (kg)	Dressing %	Moisture %	Protein %	Ash %
Buck (N= 5)	29.5 ± 1.83	16.18 ± 0.59	54.84 ± 0.31	74.29 ± 0.22	22.06 ± 0.47	1.10 ± 0.09
Castrated male (N = 3)	27.6 ± 0.86	14.85 ± 0.42	53.81 ± 0.41	74.22 ± 0.11	22.40 ± 0.14	1.09 ± 0.11
Doe (N = 14)	22.4 ± 1.03	11.08 ± 0.17	49.48 ± 0.69	74.39 ± 0.23	22.06 ± 0.15	1.12 ± 0.10

The hot carcass yield (on full stomach basis) of doe, castrated male and buck of crossbred goat of Natore has been presented in Table 13. The total meat yield and dressing% was higher in crossbred goat than those of Black Bengal goat in Natore, but was lower than those of Black Bengal goat in Bandarban Hill district.

Table 14. Carcass yield, dressing% and proximate analysis of crossbred goats in Natore

Sex	Live weight (kg)	Hot carcass yield (kg)	Dressing %	Moisture %	Protein %	Ash %
***Buck (N= 6)**	38.5 ± 1.23	18.42 ± 0.70	49.18 ± 0.51	74.49 ± 0.20	22.06 ± 0.17	1.10 ± 0.07
Castrated male (N = 2)	24.5 ± 0.26	16.15 ± 0.62	45.51 ± 0.36	74.22 ± 0.12	22.40 ± 0.13	1.09 ± 0.12
Doe (N = 10)	24.9 ± 0.98	10.66 ± 0.57	42.85 ± 0.96	74.49 ± 0.20	22.06 ± 0.17	1.22 ± 0.11

*Culled breeding bucks

The pH of the meat ranged from 5.8 to 6.2. The deep loss ranged from 1.5 to 2%. In proximate component analysis, moisture, crude protein and ash ranged from 74.49 to 74.49%, 22.06 22.40 and 1.09 to 1.22% respectively. In sensory test of cooked meat, the meat of Grade castrated male scored the highest mark.

b. QTL study

Genotyping work

Blood samples of all bucks (6 Black Bengal and 4 Beetal), 160G_0 does, 128G_1 kids, 78G_2 kids, and 20 G_3 kids was collected. DNA has been extracted and quantified for all the samples. DNA was quantified by Nono drop spectrophotometer. The Table 14 represents the parameters considered in quantification of isolated DNA samples.

Table 15. Parameters estimated in Quantification of DNA by Nano drop spectrophotometer

Sample ID	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
QTL-04	165.7	ng/µl	3.314	1.796	1.85	1.83	DNA	50
QTL-103.	539.9	ng/µl	10.798	5.751	1.88	2.16	DNA	50
QTL-261	470.6	ng/µl	9.413	5.045	1.87	2.13	DNA	50
QTL-117	111	ng/µl	2.22	1.254	1.77	1.42	DNA	50
QTL-140	299.7	ng/µl	5.995	3.299	1.82	1.64	DNA	50
QTL-266	448.7	ng/µl	8.973	4.781	1.88	2.15	DNA	50
QTL-259	415.8	ng/µl	8.315	4.439	1.87	2.1	DNA	50
QTL-665	472.5	ng/µl	9.449	5.218	1.81	1.61	DNA	50
QTL-720	421.8	ng/µl	8.435	4.488	1.88	2.14	DNA	50
QTL-746	394.2	ng/µl	7.884	4.181	1.89	2.15	DNA	50
QTL-747	255	ng/µl	5.101	2.711	1.88	2.09	DNA	50
QTL-271	270.7	ng/µl	5.415	2.904	1.86	2.08	DNA	50

PCR amplification

It has already mentioned that PCR amplification has been started for Genotyping from May 2014. However Some PCR amplification was carried out for marker testing. So far 40 microsatellite markers were positive across the populations. This confirmed by agarose gel electrophoresis test. The markers which amplified and showed distinct bands have been selected (Plate 16). Standard used was 1000 bp in length. The length of microsatellite markers had length ranging from 150 t0 350 bp. The list of some markers that were found positive in PCR has been presented in Table 2.

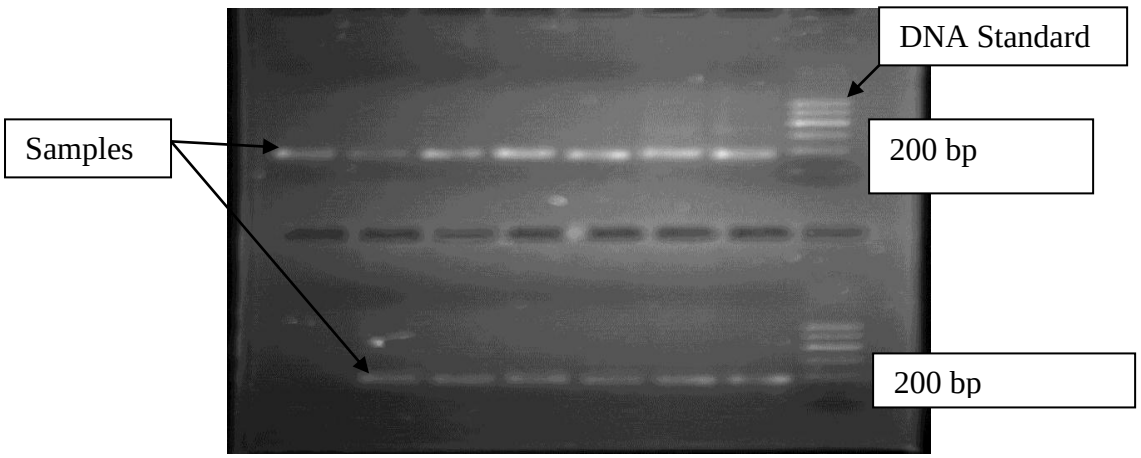

Plate 16. Band of PCR product for marker IRNA63
This marker has been selected for genotyping

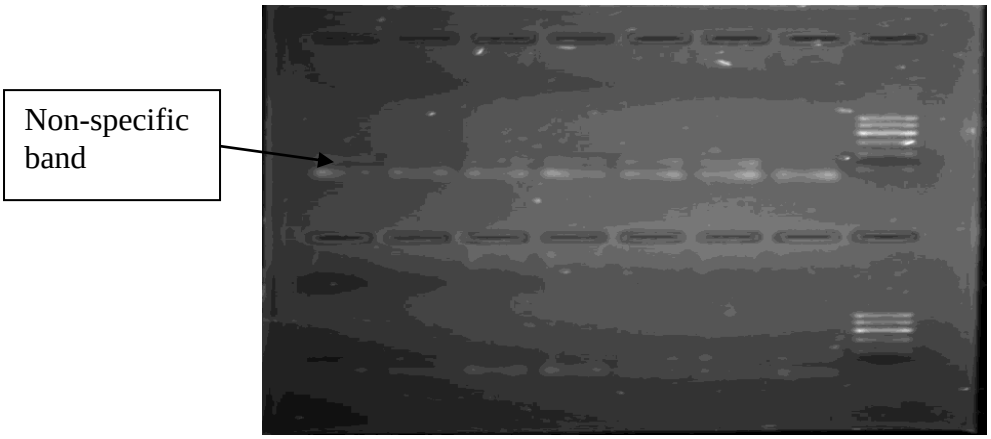

Plate 17. Band of PCR product for marker MAF 6525
This marker has rejected for genotyping due to presence of non-specific band

The QTL detection could not be completed in the schedule time of the project to lack of sufficient back cross progeny. A new mating design has been implanted in the field based on half sib mating. It will take some time, to get desired genotypes and progenies.

c. Strengthening the capacity of the Institution

The existing molecular biology laboratory of the department was renovated by procurement of some valuable equipment from the project fund. This laboratory is now being used by the under graduate and post graduate students of the department in addition to project research work.

Two Research Fellows of this project have already obtained MS degree in Animal Breeding and Genetics. Their thesis are have been submitted to concerned Department with copy to university Library. One Ph. D. Fellow and one M.S. Fellow are continuing their study.

D. Other as part of goat of the project

Economic benefit/farmer benefit/cost-benefit/ Social impact

The goal of this study was to increase the income of targeted beneficiary group, specially the women of the small and marginal farmers in order to enhance the nutritional and food security status. The following achievement could be attained to fulfill this goal.

1. The introduction of disease preventive measures and use of alternate feeds resulted in the reduction of goat mortality of enlisted farmers/ contact farmers, increased the number of goat/farmer household and also increased the income of the farmers. This is evidence from the following Figures.

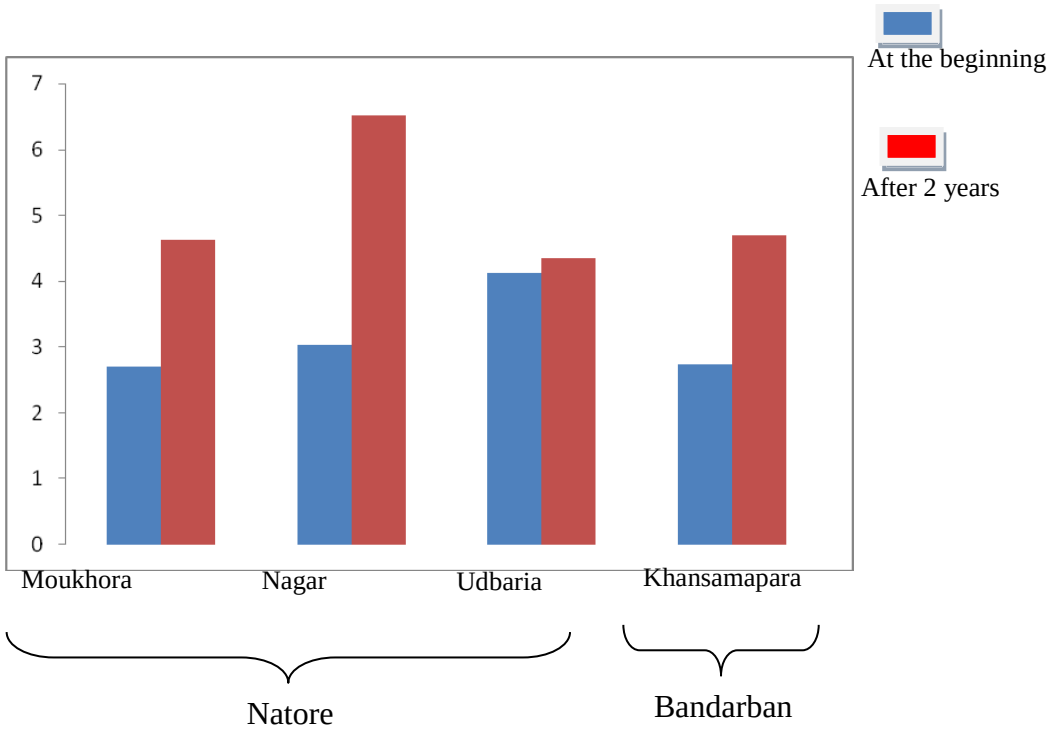

Fig. 10: Average number of goat per house-hold at the beginning of the study and after 2 years

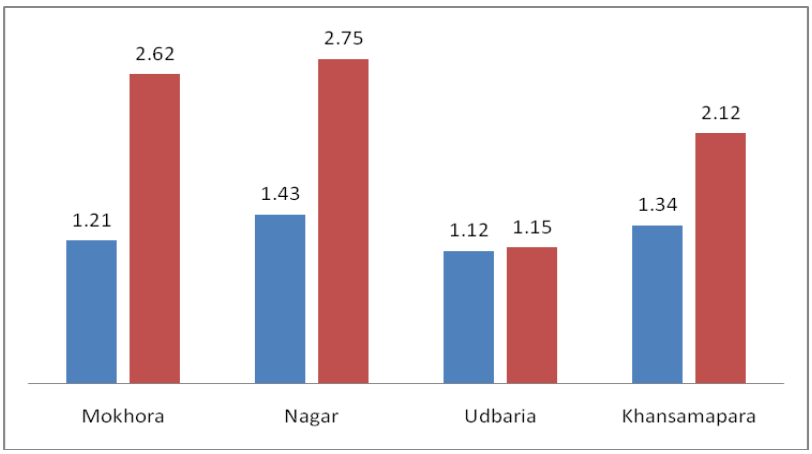

Fig. 11: Average number of goat sold by farmers at the beginning of the study and after 2 years

Case – I

Mr. Mashiur Rahman (Plate 19) is an unemployed youth and a farmer working as a volunteer in the experimental site of Natore. At the start of this program, he owned 9 goats. In the last 2 years, he was trained on record keeping and improved husbandry practices of Black Bengal goat. He sold 16 goats in last 2 years and earned 45,000.00 Taka (Gross income). He possesses 14 goats at present.

Plate 18. Mashiur Rahman with his goats

Case – II

Miss. Esha ching (Plate 20) is an unemployed girl and a farmer working as a volunteer in the experimental site of Bandarban. At the start of this program, the girl owned 5 Black Bengal goats. She obtained training on improved husbandry practice of Black Bengal goat. During the last 2 years. She could expand her flock. She sold 14 goats in the last 2 years and earned 36,000.00 (Gross income) Taka. She now possesses 10 goats.

Plate 19. Esha Ching with her goats

2 Better livelihood through meeting the needs of family member like education, primary health care etc.

3 More confidence for goatery leading self employment

4 More cooperation among the members for various social activities

Environmental Impact

The environmental monitoring report has been presented in Table 13 and Table 14.

Table 16. Environmental monitoring matrix in April, 2010

Sl. No	Environmental issue	Component	Baseline	Degree of Impact*				Remarks
				Small	Moderate	Large	None	
1	***Biodiversity***	Flora	0				0	
		Fauna	0				0	
		Genetic diversity	+ 45		+ 45			
		Exotic varieties	+ 20	+ 20				
		Local varieties/ cultivars	+ 60			+ 60		
		Hybrids	0				0	
2	***Soil quality***	Organic matter	+ 60			+ 75		
		Chemical fertilizer use	0				0	
		Soil salinity	0				0	
		Fertility status	+ 50		+ 50			
		Microbial activity	+ 20	+ 20				
		Heavy metal contamination	0				0	
		Water quality	0				0	
3	***Agro-Chemicals***	Pesticide use	0				0	
		POPs	0				0	
		IPM	0				0	
		Pest infestation	0				0	
		Bio-pesticides	0				0	
		Health hazard	0				0	
4	***Pollution***	Soil	0				0	
		Water	0				0	
		Air	0				0	

.

Table 17. Environmental monitoring matrix in March, 2014

Sl. No	Environmental issue	Component	Baseline	Degree of Impact*				Remarks
				Small	Moderate	Large	None	
1	***Biodiversity***	Flora	0	+20				
		Fauna	0	+20				
		Genetic diversity	+45			+50		
		Exotic varieties	+20		+30			
		Local varieties/ cultivars	+60			+60		
		Hybrids	0				0	
2	***Soil quality***	Organic matter	+75			+80		
		Chemical fertilizer use	0				0	
		Soil salinity	0				0	
		Fertility status	+50			+60		
		Microbial activity	+20		+30			
		Heavy metal contamination	0				0	
		Water quality	0				0	
3	***Agro-Chemicals***	Pesticide use	0				0	
		POPs	0				0	
		IPM	0				0	
		Pest infestation	0				0	
		Bio-pesticides	0				0	
		Health hazard	0				0	
4	***Pollution***	Soil	0				0	
		Water	0				0	
		Air	0				0	

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11. Research Highlights:

i. This experiment was carried out in Bangladesh Agricultural University, Mymensingh, Natore and Bandarban Hill district to produce breeding stock of Black Bengal goat and to detect Quantitative Traits Loci (QTL) of economic traits

ii. Black Bengal goats (Bucks and does) with outstanding genetic merit have been produced in Bandarban Hill district.

iii. Back cross goats could not be produced in Natore district as part of requirement of the experiment.

iv. An easy and economic system for animal recording of goat reared under semi intensive system has been developed. This is participatory farming system of Black Bengal goat for genetic improvement. **This is the most important technology that has have generated in this project.**

v. The disease prevention measures for goats reared under intensive system have also been standardized. This is another important knowledge we have generated in this project. This has resulted in increasing the income of goat farmers possessing Black Bengal goats 1.5 to 2.0 times more.

vi. The genotyping study for QTL study is in progress. When finished, it will provide tools and techniques for application of biotechnology in goat breeding program in Bangladesh

vii. The existing molecular genetics laboratory has been modernization. **It has strengthened the teaching and research of the department in molecular genetics**

12. Major Attainments *(in relation to the set objectives)* :

a. Technical: Output, Outcome and Impact

Sl. No	Major technical activities performed in respect of the set objectives	Output(i.e product obtained, visible, measurable)	Outcome(short term effect of the research)	Impact(long term effect of the research)	Remarks (reason, if anything otherwise plus any other)
1a	Production of pure Black Bengal bucks, crossbred and back cross does	Superior Black Bengal goats (8 Bucks and 40 does) have been produced in Bandarban Hill district	Availability of outstanding Black Bengal goats with higher genetic merit	Enhancement of genetic improvement of Black Bengal goat	None
		Production of 304 crossbred goats	Comparative performance of Black Bengal and crossbred under rural condition	Fitness of crossbred goats under rural condition could be	

				tested	
		24 Back cross progenies are being produced in Natore	QTL study will be possible	back cross goat reared under semi intensive system can be tested	
1b	Development of easy and economic system for animal recording of goat reared under semi intensive system	Identification of breeding stocks and proper selection of dam and sire	Goat breeding program can be run for goats reared under semi intensive system	Genetic improve of Black goat reared under semi intensive system will be possible	
1c.	The disease prevention measures for goats reared under intensive system have also been standardized	More goat number/ farmer	Income of farmers has increases 1.5 to 2.0 time more	Goat farms will be profitable	
2	Identifying the Quantitative Traits Loci (QTL) in Black Bengal goat for meat quantity and quality and other economic traits	Back cross progenies are available DNA have been extracted from G_1 (pure Black Bengal dam and sire), G_2 (cross bred dam and sire) and G_3 back cross progenies. Microsatellites markers have been tested for these populations	Availability of pedigree flock of goats of different genetic combination Detection of QTL will be possible	Study of functional genomics like QTL, GWAS will be possible	Delay in production of back cross progenies hampered detection of QTL
3	Enhancing the institutional capacity for education and research	Procured the required equipments for genotype	The post graduate students of this department and other departments are using these equipment for conducting their research regularly	The teaching and research quality in the field of molecular genetics and biotechnology will be improved	None

b. Procurement

Sl. No	Approved provisions of Procurement (list major items)	Achievement	% of achievement	Remarks (reason, if anything otherwise)
A	**Lab equipment**			All the machine is in active condition and are being used by the post graduate students of the department and university
	Adjustable Multi channel pipette, 8 tube capacity (0.2-10 µ l) 1 no.; Eppendorf	Procured one	100%	
	-Bio Doc –It system for gel imaging for PCR products, with camera and monitor fitted with UV ray. UVP, Uk	Procured one	100%	
	-NanodropUV-Vis spectrophotometer 2000 wave length 190-840; for DNA, RNA and protein quantification. NanoDrop products, USA	Procured one	100%	
	-Centrifuge with swing bucket rotator with adoptor, 4400 rpm, Eppendorf, Germany	Procured one	100%	
	- Microtome, thickness setting 0.5-60 µm, Sakura, Japan or China			
	-ThermoMixer Comfort (Heat, cool & mixed) with 24 x 1.5 ml thermoblock (4-90 0C) 300 rpm	Procured one	100%	
	-Pipette stand carousel for 6 micropipette	Procured one	100%	
	-			
B.	**Office equipments and/or furniture**			
	1.Computer Desktop, accessory (Laser printer etc) and **software** (Gene mapper, Gene scan etc) for QTL and sequence data analysis	Procured three	100%	
	2. Heavy duty IPS for laboratory to supply electricity during load shading	Procured two	100%	
	3.Furniture	NA	100%	
	4.Motor cycle	Procured one	100%	
	5. Bi-cycle	Procured five	100%	

c. HRD/ Training

Title (e.g Ph.D/MS/ Trainings, workshops conducted etc.)	Target	Attainments	No. of participants	Benefit of the higher studies/trainings(application of the learning, productivity enhancement	Remarks (reason, if anything otherwise)
Ph.D.	1 No	Research work completed, Thesis will be submitted within August, 2015 as per university rule	1 No	The student will be able to assist in implementation of goat breeding program in future	Draft copy of thesis will be submitted by June, 2014
MS	3 No	2 No	3 No	The students will be able to assist in implementation of goat breeding program in future	1 MS student is in study. Thesis will be submitted by November, 2014
Training of farmers	150 No	150 No	150 No	It assisted in running the program smoothly	
Workshop	1	1	80 No	Participant included policy makers and researchers from all stake holders. So goat breeding concept was recognized.	It was a national symposium
Farmer days	-	3	180 No	Farmers from different units united together and exchange their experience about goat rearing and breeding	
Farmers volunteers	-	15 No	15 No	They are trained in primary treatment and preventive measures of goat disease as well as animal recording thus making possible to run the goat breeding program	**This is additional achievement**

d. Financial

Sl. No	Major Head	Fund received (Tk.)	Expenditure (Tk.)	Balance/Unspent (Tk)	Remarks (reason, if anything otherwise)

1	Salary & Remuneration	2561320	2561320	0	
2	Research Expenses	5725787	5725787	0	
3	Operating Expenses	812014	812014	0	
4	Fuel, Oil and Maintenance	137401	137401	0	
5	Training/Seminar etc.	390000	390000	0	
6	Publication & Printing	300000	300000	0	
7	Contingencies	312699	312699	0	
8	Capital Expenses	3384599	3384599	0	
9	**Total**	**1,36,23,820.00**	**1,36,23,820.00**	**0**	

e. Materials developed/Publications made:

Type of material/publication	**Title**	**Number**	**Remarks(being used by/meant for/any other)**
Technology development	Animal recording system for goat reared under semi intensive system Protocol for application of biotechnology in goat breeding and constraints in implementation of this protocol in Bangladesh	1	A number of NGO has expressed their willingness to adopt and implement this technology
Process development	Selective breeding for goat reared under semi intensive system	1	A number of NGO has expressed their willingness to adopt and implement this technology
Information development	Participatory goat breeding system for genetic improvement of Black Bengal goat	1	Not yet
Journal publication	None		2 manuscripts are Under preparation.
Books/Monographs/Manual published	*One manual* ছাগল খামারী ও মাঠকর্মীর জন্য ভাল ছাগল ও পালন নির্দেশিকা	500	Are being used by Farmers
Booklet/leaflet/flyer etc. published	*One Newsletter and One booklet* Participating farming technique of pure Black Bengal goat for genetic improvement	500	Are being used by academician, researchers and farmers
Any other (patenting of technology etc.)	None	-	-

13. Sub-project Auditing

Types of Audit (e.g BARC/Implementing agency/FAPAD/World Bank/others)	**Major observations/issues/objections raised, if any**	**Status at the sub-project end**	**Remarks**
BARC-PUC	No objection	Satisfactory	
FAPAD	No objection	Satisfactory	
J & U Co.	No objection	Satisfactory	

14. Reporting

Report type	**Actual date of submission(s)**	**Total Number(s)**	**Remarks(if anything otherwise)**
a. Inception report	1st week of May,2010	2	
b. Monthly reports*	1st week of every month	41	
c. Statement of expdts.(SoE)*	1st week of every month	41	
d. Quarterly report(s)*	July 10, Oct,10, Feb, 11; Aril 11; August 11, Dec 11, Jan 12	7	
e. Six monthly report	Dec 10, Jan 12 Dec 12	3	
f. Procurement plan	19.5.10	1	
g. Annual research program format	August 10, July 11 , July 12	3	
h. Environmental monitoring (Annual Basis)	May, 12 Nov, 12	2	
i. Social safeguard status (Before and at the end)	Three at a time	2	
j. Field Monitoring Report(s)**	June11, Nov.11, December11	3	The team did not visit field further

** Provide all since start to end.*

*** Conducted at the local level by implementing agencies.*

15. Problem / Contraints

- The production of back cross progress delayed due to loner puberty age of crossbred goat. Some of the crossbred goat was not even responded to Assisted Reproductive Technique like hormone treatment.
- That delayed in production of backcross progenies in required number of this experiment thus hampered in detection of QTL.
- At the same, mortality rate of crossbred goats due to disease is also higher under semi intensive system

16. Suggestion for future, if any :

- *This study indicates that crossbred goats produced in the vast area of North Western parts of country require new management and environment for the successful farming with crossbred goats. The genetic abnormalities like chromosomal abnormalities should also be investigated*

Prof. Dr. Md. Omar Faruque
Principle Investigator

Prof. Dr. Lutful Hassan
Director, BAURES